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Microbiological response and treatment outcomes among multidrug-resistant tuberculosis patients at Allied Hospital, Faisalabad, Pakistan: A prospective cohort study

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Abstract

Despite being a long-term disease, treatment success and increasing antimicrobial resistance, multidrug-resistant tuberculosis (MDR-TB) continues to pose a significant public health threat, especially in low-resource countries. The present study aimed to assess the microbiological outcome and the treatment efficacy of MDR-TB patients in Allied Hospital, Faisalabad, Pakistan, with special regard to sputum culture conversion. A prospective cohort study was conducted at Allied Hospital in Faisalabad, Pakistan, from February 2025 to August 2025 at the MDR-TB Unit. The sample was obtained using a non-probability consecutive sampling technique, and 100 confirmed adult patients of MDR-TB were enrolled and followed throughout the treatment. Demographic and clinical data were collected at baseline; microbiological response was monitored by culture on Lowenstein-Jensen medium from sputum samples taken at regular intervals. The data were then processed with descriptive statistics with the help of SPSS software version 26.0. Of the patients enrolled, 62% were male, and 72% had a previous TB treatment history. Sputum culture conversion was seen in 32% of patients in the first two months, 41% from three to four months, 17% from five to six months, and 10% after six months. Overall, 48% of patients were cured,

27% completed treatment, 15% failed treatment, and 10% died. Sputum culture conversion was seen to be associated with a favorable outcome of treatment, with delayed culture conversion seen to be more associated with treatment failure and death. These findings indicate that regular microbiological monitoring could help to timely assess treatment response and provide better clinical management for MDR-TB. The results obtained should be confirmed by further multicenter studies with larger numbers of patients.

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Introduction

Tuberculosis (TB) remains one of the most significant infectious diseases worldwide despite considerable advances in its diagnosis, treatment, and prevention. It is caused by Mycobacterium tuberculosis, an obligate aerobic acid-fast bacillus that primarily affects the lungs but may also involve extrapulmonary sites, including the lymph nodes, bones, kidneys, and central nervous system. TB is transmitted through airborne droplets released when infected individuals cough, sneeze, or speak, making it highly contagious, particularly in overcrowded and resource-limited settings [1].

According to recent global estimates, tuberculosis continues to impose a substantial public health burden, particularly in low- and middle-income countries. South Asia remains one of the regions most affected by TB, with Pakistan ranking among the countries with the highest burden of tuberculosis and multidrug-resistant tuberculosis (MDR-TB). Poverty, malnutrition, overcrowding, HIV infection, diabetes mellitus, and limited access to healthcare services are important factors contributing to the persistence and transmission of the disease [2].

Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin, poses a major challenge to global TB control. Management of MDR-TB requires prolonged treatment with second-line anti-tuberculosis drugs that are less effective, more toxic, and considerably more expensive than standard first-line [illegible] associated with lower treatment success rates, increased mortality, and greater healthcare costs [3]. The World Health Organization (WHO) recommends early diagnosis using rapid molecular techniques, prompt initiation of appropriate treatment, and continuous microbiological monitoring to improve treatment outcomes and reduce disease transmission [4]. The multi-faceted nature of MDR-TB has been associated with gaps and deficiencies in treatment, treatment outcomes, drug compliance, drug selection, drug supply disruptions, and health systems. Further, the emergence and progression of drug resistance are influenced by biological factors, such as high bacterial load and genetic mutations in the pathogen, as well as host factors such as immune status. These pressures make it difficult to manage the disease clinically and also enable resistant strains to continue to spread in communities [5]. Diagnosis and treatment planning require confirmation of rifampicin and isoniazid resistance for a proper diagnosis and treatment of MDR-TB.

Treatment for MDR-TB is very different than treatment for drug-susceptible TB. It demands treatment with second-line anti-tubercular drugs, which are less effective, toxic, and much more costly. There are a variety of side effects with treatment regimens that are often prolonged (9 to 20 months or more) and include hepatotoxicity, nephrotoxicity, ototoxicity, and neurotoxicity [6]. All these factors play a role in poor adherence, higher chances of treatment failure, and deaths.

The diagnosis and treatment of tuberculosis have been revolutionized in recent years with the development of new technologies for diagnosis. Sputum smear microscopy and culture are still basic tests, and culture is still considered the gold standard for confirming infection and checking the response to treatment. These approaches, however, are time-consuming and can cause delays in vital clinical decisions. The advent of fast molecular diagnostic tests, including the GeneXpert MTB/RIF test, has greatly improved the capacity to detect MTB and rifampicin resistance in a few hours [7]. Notwithstanding these developments, the use of molecular diagnostics and traditional culture-based approaches will continue to be vital for thorough patient assessment and surveillance. Sputum culture conversion has been one of the most reliable and clinically meaningful parameters to assess treatment effectiveness in MDR-TB patients, among other indicators [8].

Pakistan remains among the countries with the highest burden of tuberculosis and MDR-TB worldwide. Socioeconomic challenges, delayed diagnosis, limited healthcare resources, treatment interruption, and poor adherence continue to affect disease control and treatment outcomes [9, 10]. Allied Hospital, Faisalabad, is a major tertiary care referral center providing specialized diagnostic and treatment services for MDR-TB patients from Faisalabad and surrounding districts. Despite its important role in MDR-TB management, limited local data are available regarding microbiological response and treatment outcomes among patients receiving routine programmatic care. Generating such evidence is essential for evaluating the effectiveness of current treatment strategies and identifying areas requiring improvement [11].

Although several demographic and clinical factors, including age, sex, nutritional status, HIV infection, diabetes mellitus, and previous TB treatment, have been reported to influence MDR-TB treatment outcomes, microbiological response remains one of the most reliable indicators of treatment success. Assessing sputum culture conversion during treatment may facilitate early identification of patients at risk of unfavorable outcomes and support timely clinical interventions [12].

While microbiological factors are important, there are also several patient-related and clinical variables that have been found to affect outcomes of MDR-TB treatment. These factors include age, gender, nutritional status, HIV infection, diabetes, history of past TB treatment, and compliance with therapy. Socio-economic factors such as educational attainment, income, and availability of health care services also have a significant influence on treatment outcomes [13]. Thus, laboratory-based indicators and patient-specific ones should both be taken into account for a thorough assessment of the effectiveness of treatment for MDR-TB.

Therefore, the present study was conducted to evaluate the microbiological response and treatment outcomes among patients with multidrug-resistant tuberculosis receiving treatment at Allied Hospital, Faisalabad, Pakistan. Particular emphasis was placed on sputum culture conversion and its association with treatment outcomes to provide evidence that may support improved patient management and strengthen regional tuberculosis control strategies.

Materials and Methods

Study design

This prospective cohort study was conducted at the Multidrug-Resistant Tuberculosis (MDR-TB) Unit, Allied Hospital, Faisalabad, Pakistan, from February 2025 to August 2025. The study was designed to evaluate sputum culture

conversion and its association with treatment outcomes among patients diagnosed with MDR-TB. Ethical approval was obtained from the Institutional Review Committee of Allied Hospital before the commencement of the study.

Study population

A total of 100 consecutive adult patients with confirmed MDR-TB were enrolled during the study period using a non-probability consecutive sampling technique. All eligible patients presenting to the MDR-TB Unit during the study period who fulfilled the inclusion criteria were invited to participate. MDR-TB was confirmed by the GeneXpert MTB/RIF assay and hospital MDR-TB treatment records.

Data collected included age, sex, previous tuberculosis treatment history, sputum culture status, time to culture conversion, and final treatment outcome. Information regarding HIV status, diabetes mellitus, body mass index, smoking history, cavitary disease, and extended drug susceptibility testing was not routinely available and was therefore not included in the analysis.

Sample size

Because this was a hospital-based prospective cohort study, all eligible MDR-TB patients attending the MDR-TB Unit during the study period who fulfilled the inclusion criteria were consecutively enrolled. Therefore, no formal sample size calculation was performed

Inclusion criteria

1. Confirmed MDR-TB patients diagnosed by GeneXpert MTB/RIF assay.
2. Patients aged 18 years and above.
3. Patients willing to participate and complete follow-up.

Exclusion criteria

1. Patients with extrapulmonary tuberculosis.
2. Patients receiving anti-tuberculosis treatment for less than two weeks.
3. Pregnant women.
4. Patients younger than 18 years.

Treatment regimen

Patients received individualized MDR-TB treatment according to the National Tuberculosis Control Program (NTP) Pakistan guidelines, consistent with the World Health Organization recommendations. Treatment regimens were selected on the basis of rifampicin resistance, previous treatment history, clinical assessment, and drug availability. Drug dosages were adjusted according to body weight, and patients were monitored regularly for adverse drug reactions, treatment adherence, and microbiological response throughout the treatment period.

Materials and reagents

The materials used during the study included sterile sputum collection containers, disposable gloves, N95 masks, laboratory coats, microscope slides, immersion oil, Ziehl–Neelsen staining reagents (carbol fuchsin, acid alcohol, and methylene blue), Lowenstein–Jensen (LJ) culture media, GeneXpert MTB/RIF cartridges, centrifuge tubes, NALC-NaOH decontamination reagents, sterile pipettes, disinfectants, and distilled water.

The instruments used were a GeneXpert MTB/RIF system, a Class II biosafety cabinet, an incubator, a centrifuge machine, a refrigerator, an autoclave, a vortex mixer, an analytical balance, micropipettes, a light microscope, and laboratory glassware.

Sample collection

Sputum samples were taken from all the patients enrolled under aseptic conditions. Sputum samples were collected in leak-proof, sterile containers early in the morning. The baseline samples were taken before treatment for MDR-TB began, and follow-up samples were taken at regular intervals in line with the national MDR-TB treatment guideline. All samples were labeled properly and sent to the microbiology lab for further processing.

Sample processing

Sputum samples were collected and processed by the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) digestion and decontamination process. The samples were then thoroughly mixed with the decontamination solution and left to stand for the recommended time. All specimens were then decontaminated and centrifuged after adding phosphate buffer to them. The sediment collected was used for smear microscopy, GeneXpert testing, and culture, and the supernatant was discarded.

Ziehl–Neelsen staining

Direct smear microscopy was done with the Ziehl-Neelsen staining technique. A thin smear was made on a clean glass slide, air-dried, and then heat-fixed. The slide was flooded with carbol fuchsin and gently heated until steaming. After washing with water, the smear was decolorized using acid alcohol and subsequently counterstained with methylene blue. The stained slides were examined under oil immersion (100×) using a light microscope to detect acid-fast bacilli (AFB).

GeneXpert MTB/RIF assay

The GeneXpert MTB/RIF assay was performed according to the manufacturer's instructions. Processed sputum samples were mixed with the sample reagent and loaded into GeneXpert cartridges. The assay simultaneously detected Mycobacterium tuberculosis complex and rifampicin resistance. The National Tuberculosis Control Program (NTP) Pakistan guidelines state that diagnostic data from hospital MDR-TB treatment records and the NTP registry were used to classify MDR-TB. When choosing a course of treatment, national guidelines for managing drug-resistant tuberculosis were carefully followed.

Sputum culture

The processed sputum sediment was inoculated onto Lowenstein–Jensen (LJ) culture medium under aseptic conditions. The inoculated media were incubated at 37°C and examined weekly for growth. Cultures were monitored for up to eight weeks. The appearance of characteristic rough, buff-colored colonies was considered suggestive of Mycobacterium tuberculosis growth. Negative cultures were confirmed after completion of the incubation period.

Identification of Mycobacterium tuberculosis

Positive cultures were identified based on colony morphology, acid-fast staining characteristics, and correlation with GeneXpert MTB/RIF results. The presence of acid-fast bacilli on microscopy, together with positive culture findings, confirmed Mycobacterium tuberculosis infection.

Sputum culture conversion

Sputum culture conversion was defined as two consecutive negative cultures obtained at least 30 days apart after initiation of MDR-TB treatment. Time for culture conversion was noted and grouped by length of treatment.

Outcome measures

The main endpoint of the study was the microbiological response to MDR-TB treatment, measured by sputum culture conversion. Secondary outcomes were cure, treatment completion, treatment failure, and death. These outcomes were assessed as per usual MDR-TB treatment guidelines and used to assess the overall effectiveness of treatment and its relationship to culture conversion status.

Statistical analysis

Data were entered and analyzed using IBM SPSS Statistics version 26.0. Continuous variables were summarized as mean ± standard deviation (SD), whereas categorical variables were expressed as frequencies and percentages. Because the available dataset was primarily descriptive and lacked complete patient-level covariates required for inferential modelling, descriptive statistical analysis was performed to summarize demographic characteristics, sputum culture conversion patterns, and treatment outcomes.

Results

Demographic characteristics

100 patients with multidrug-resistant tuberculosis (MDR-TB) were followed during their entire treatment course to assess microbiological response and outcomes in the present study. The demographic profile showed that there was a significant difference in the number of male and female patients, with males making up 62% and females 38% of the study population. This gender imbalance indicates that males might be more likely to be exposed to risk factors, e.g., work-related exposures, smoking, and delayed care-seeking, that could contribute to the higher burden of MDR-TB seen among males.

Table 1. Baseline demographic characteristics of the study participants (n = 100)

Characteristics	Number	%age
Male	62	62
Female	38	38
Previous TB treatment	72	72
No previous TB treatment	28	28

Age-wise, it was observed that the disease affected a wide age group, but most of the patients were in the economically productive age group. The age distribution of the study participants showed that the highest proportion of patients belonged to the 31–40-year age group (33%, n = 33), followed by the 18–30-year age group (28%, n = 28) and the 41–50-year age group (23%, n = 23). Patients aged ≥51 years constituted the smallest proportion of the study population (16%, n = 16). This trend suggests that the major proportion of MDR-TB patients are young and middle-aged adults, which can have important socioeconomic consequences as a result of productivity loss and healthcare burden.

Microbiological findings

At baseline, all enrolled patients had microbiologically confirmed MDR-TB with positive sputum culture and rifampicin resistance detected by the GeneXpert MTB/RIF assay. Serial sputum cultures were performed throughout treatment to evaluate microbiological response.

Sputum culture conversion

All enrolled patients were confirmed to have active disease and resistance to 1st line therapy, as they had all been confirmed to be sputum culture positive and resistant to rifampicin. Sputum samples were serially cultured during

treatment, and different culture conversion patterns were observed. An early and favorable microbiological response was noted with approximately 32% of patients achieving culture conversion within the first two months of therapy. Forty-one percent of the patients were converted between the 3rd and 4th month, and 17% needed 5-6 months. In particular, 10% of patients had delayed conversion (more than 6 months after treatment), suggesting a slow response to treatment and the possibility of adverse treatment effects (**Fig. 1**).

Treatment outcomes

According to WHO treatment outcome definitions, 48% of patients were classified as cured, 27% completed treatment, 15% experienced treatment failure, and 10% died during the treatment period. The results highlight the complexity and difficulties of treating MDR-TB, especially in resource-limited areas (**Fig. 2**).

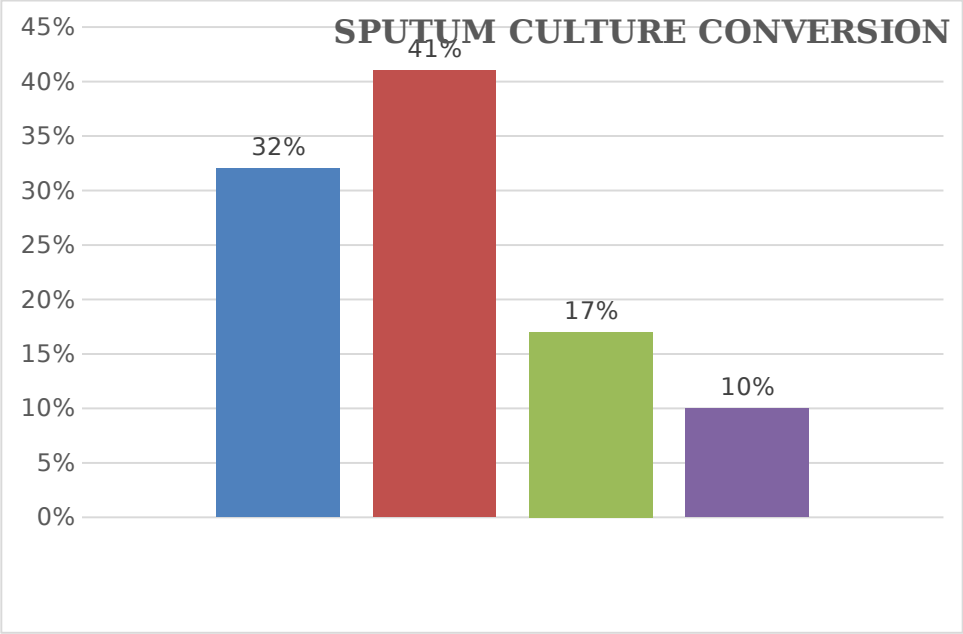

Fig. 1: Sputum culture conversion status among patients during the treatment period

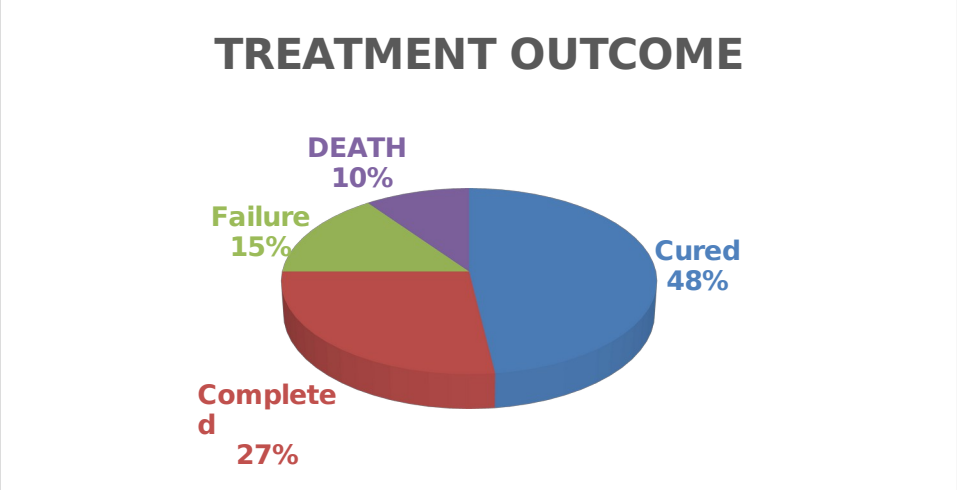

Fig. 2: Treatment outcomes of patients following management and follow-up

Patients with early culture conversion (1–2 to 4 months) tended to have better outcomes, including cure or treatment completion. Patients who had a late culture conversion had a higher proportion of treatment failure and death, on the other hand. Based on these observations, there is a possibility that culture conversion can be used as an important clue to treatment outcome with MDR-TB patients.

Discussion

In the present study, we assessed sputum culture conversion and the outcome of treatment in 100 patients having multidrug-resistant TB (MDR-TB). The results showed that there was a predominance of male patients (62%), and the highest disease burden was seen in the age group 31-40 years. These findings have been observed in several studies conducted in high-burden countries where the prevalence of MDR-TB is higher among males and those of economically productive age, as they are exposed more often to environmental risk factors, occupational risk factors, smoking, and take too long to seek medical treatment. MDR-TB is concentrated in the working-age population, indicating the significant socioeconomic burden of MDR-TB [14].

Sputum culture conversion continues to be one of the most critical markers of response to treatment for MDR-TB. In the present study, 32% of the patients had converted their culture in the initial two months of treatment, and the majority (41%) had converted in the third to fourth month of treatment. These findings are similar to the previous studies, which have reported the majority of MDR-TB patients as culture converting in the first four months of effective treatment [15]. Early conversion has always been found to be a good prognostic indicator and a correlation with a favorable treatment outcome and lower bacterial burden.

The results of the treatments in this study showed that 48% of patients were cured, 27% finished the treatment, and 27% had favorable results overall. But 15% of patients failed to respond to the treatment and 10% died. The same kind of results have been seen in other studies carried out in resource-limited areas, where factors like delayed diagnosis, adverse drug reactions, treatment non-adherence, long chains of drug resistance, and others can lead to poor outcomes [16]. The rate of success seen in the current study is promising, but still not as high as those set by the global tuberculosis control programs.

Sputum culture conversion was associated with treatment outcomes in the present study. In the present cohort, earlier sputum culture conversion was associated with more favorable treatment outcomes, whereas delayed conversion was observed more frequently among patients with treatment failure or death. Because only descriptive analyses were performed, these findings should be interpreted as an association rather than evidence of a causal or predictive relationship. Further studies using multivariable statistical analyses are required to confirm this observation [17]. Thus, routine microbiological monitoring is necessary for the detection of patients at risk of poor outcomes and for timely clinical interventions.

The GeneXpert was a crucial tool in the swift identification of rifampicin resistance in the participants of the

study. But there was still no alternative to culture testing for monitoring treatment response and treatment success. This finding aligns with the current international recommendations to take culture conversion as the gold standard to assess therapeutic effectiveness in the treatment of MDR-TB [18].

These findings support the importance of early diagnosis, routine microbiological monitoring, and timely clinical management in patients with MDR-TB. Nevertheless, larger multicenter prospective studies incorporating detailed clinical variables and multivariable statistical analyses are required to validate these observations and strengthen the evidence.

This study has several limitations. First, it was conducted at a single tertiary care center with a relatively small sample size, which may limit the generalizability of the findings. Second, non-probability consecutive sampling may have introduced selection bias despite standardized patient enrolment procedures. Third, detailed baseline clinical variables, including HIV status, diabetes mellitus, body mass index, smoking history, cavitary disease, adherence assessment, and comprehensive drug susceptibility testing beyond rifampicin resistance, were unavailable and therefore could not be evaluated. Finally, inferential statistical analyses such as logistic regression, Cox proportional hazards modelling, or Kaplan–Meier analysis could not be performed because the available dataset contained only descriptive variables. Consequently, the observed relationship between sputum culture conversion and treatment outcomes should be interpreted as an association rather than evidence of prediction or causation.

Conclusion

In this prospective cohort of patients with multidrug-resistant tuberculosis, earlier sputum culture conversion was associated with more favorable treatment outcomes, whereas delayed conversion was observed more frequently among patients with treatment failure or death. Routine microbiological monitoring may assist clinicians in evaluating treatment response during follow-up. However, because the present study was based primarily on descriptive analyses, these findings should be interpreted cautiously. Larger multicenter studies incorporating comprehensive clinical variables and inferential statistical analyses are warranted to further validate these observations.

Sputum culture conversion should be monitored routinely to evaluate response to treatment and identify patients who have inadequate treatment response early. Early detection of non-responders can guide timely modification of treatment, leading to better clinical results and minimizing the risk of drug resistance. Regular counselling and follow-up visits are required to improve adherence of the patient and for them to complete their therapy. Moreover, the increase in the number of rapid diagnostic centers is essential to the early detection of the disease and the timely initiation of treatment. Improving tuberculosis (TB) control, especially in high-burden countries, is also required to limit the spread of the disease and enhance the general public health results.

Conflict of interest

The authors declare no conflict of interest.

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